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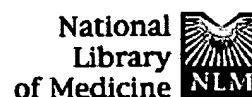
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FHL2 (SLIM3) is not essential for cardiac development and func**Chu PH, Bardwell WM, Gu Y, Ross J Jr, Chen J.**Department of Medicine, School of Medicine, University of California at San D
La Jolla, California 92093-0613, USA.

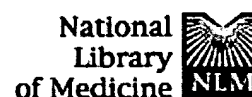
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LIM domain-containing proteins play critical roles in vertebrate development at cellular differentiation. Recently, four members of the four and one-half LIM pr (FHL) family have been identified and cloned. One of these, FHL2, is expressed in a restricted manner in the cardiovascular system throughout development into adulthood, suggesting that FHL2 may play an important role in cardiovascular development and function. Here we describe the generation and analysis of mice carrying a null mutation of the FHL2 gene. FHL2-deficient mice are viable and maintain normal cardiac function both before and after acute mechanical stress induced by aortic constriction. These data suggest that FHL2 is not essential for cardiac development and function.

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Protein-protein interaction of FHL2, a LIM domain protein preferentially expressed in human heart, with hCDC47.

Chan KK, Tsui SK, Ngai SM, Lee SM, Kotaka M, Waye MM, Lee CY, Fur KP.

Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong

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In the yeast two-hybrid library screening, the heart-specific FHL2 protein was found to interact with hCDC47. In vitro interaction study between FHL2 protein and hCDC47 was demonstrated. From the results of domain studies by the yeast two-hybrid assay, the second and third LIM domains in conjunction with the first LIM domain of FHL2 were identified to be important in binding with hCDC47. Besides, in Northern blot hybridization of human cancer cell lines, the highest FHL2 mRNA expression was detected in colorectal adenocarcinoma SW480 and HeLa S3. Our results imply that FHL2 protein may associate with cancer development and may act as a molecular adapter to form a multicomplex with hCDC47 in the nucleus, thus it plays an important role in the specification or maintenance of the terminally differentiated phenotype of heart muscle cells. Copyright 2000 Wiley-Liss, Inc.

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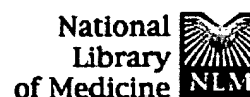
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Molecular cloning and characterization of FHL2, a novel LIM domain protein preferentially expressed in human heart.**Chan KK, Tsui SK, Lee SM, Luk SC, Liew CC, Fung KP, Waye MM, Lee**

Department of Biochemistry, Chinese University of Hong Kong, Shatin, Hong Kong

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A full-length cDNA clone encoding a novel LIM-only protein was isolated and sequenced from a human fetal heart cDNA library. This full-length clone consists of 1416 base pairs and has a predicted open reading frame (ORF) encoding 279 amino acids. The ORF of this polypeptide codes for the human heart-specific four and LIM-only protein 2 (FHL2). It possesses an extra zinc finger that is a half LIM domain and four repeats of LIM domain. When the human FHL2 cDNA probe was used to hybridize with poly-A RNA of various human tissues, a very strong signal could be seen in heart tissues, and only moderately low signals could be detected in placenta, skeletal muscle and ovary. Virtually no signal could be detected in brain, lung, liver, kidney, pancreas, spleen, thymus, prostate, testis, small intestine, colon, peripheral blood leukocyte. FHL2 was mapped to chromosome 2q12-q13 by fluorescent in-situ hybridization (FISH).

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Protein-protein interaction of FHL3 with FHL2 and visualization of their interaction by green fluorescent proteins (GFP) two-fusion fluorescence resonance energy transfer (FRET).

Li HY, Ng EK, Lee SM, Kotaka M, Tsui SK, Lee CY, Fung KP, Waye MM

Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China.

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LIM domain proteins are found to be important regulators in cell growth, cell fate determination, cell differentiation and remodeling of the cell cytoskeleton. Human Four-and-a-half LIM-only protein 3 (FHL3) is a type of LIM-only protein that contains four tandemly repeated LIM motifs with an N-terminal single zinc finger (half LIM motif). FHL3 expresses predominantly in human skeletal muscle. In this report, FHL3 was shown to be a novel interacting partner of FHL2 using the yeast two-hybrid assay. Furthermore, site-directed mutagenesis of FHL3 indicated that LIM2 of FHL3 is the essential LIM domain for interaction with FHL2. Green fluorescent protein (GFP) was used to tag FHL3 in order to study its distribution during myogenesis. Our result shows that FHL3 was localized in the focal adhesion and nucleus of the cells. FHL3 mainly stayed in the focal adhesion during myogenesis. Moreover, using site-directed mutagenesis, the LIM1 of FHL3 was identified as an essential LIM domain for its subcellular localization. Mutants have given rise to a novel technique, two-fusion fluorescence resonance energy transfer (FRET), in the determination of protein-protein interaction at particular subcellular locations of eukaryotic cells. To determine whether FHL2 and FHL3 interact with one another and to locate the site of this interaction in a single intact mammalian cell, we fused FHL2 and FHL3 to different mutants of GFP and studied their interactions using FRET. BFP/GFP fusion constructs were cotransfected in muscle myoblast C2C12 to verify the colocalization and subcellular localization by FRET. We found that FHL2 and FHL3 were colocalized in the mitochondria of C2C12 cells and FRET was observed by using an epi-fluorescent microscope equipped with an FRET specific filter set. Copyright 2001 Wiley-Liss, Inc.

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